Systematic Discovery of Function for an Uncharacterized Transcription Factor

Richard S. Hall[1], Ricardo Martí-Arbona[1]; Tuhin S. Maity[1]; John M. Dunbar[1]; Clifford J. Unkefer[1] and Pat J. Unkefer[1]

***Short Abstract* — Effectors of a putative transcription factor were selected for screening based on functional patterns within genomic proximity. Bxe_A0736 from *Burkholderia xenovorans* LB400 is located near genes involved in the oxidative tryptophan degradation pathway. From a mixture of tryptophan degradation pathway metabolites, L-kynurenine, was identified by FAC-MS and thermal-shift assays to have a high degree of affinity for Bxe_A0736. A DNA consensus binding motif for Bxe_A0736 was identified through protein binding microarray analysis, resulting in the identification of multiple possible regulatory sites. L-kynurenine dependent changes in the DNA binding of Bxe_A0736 are currently under investigation using fluorescence anisotropy.**

***Keywords* — Interactions, Protein, Ligands, DNA, Metabolism, Regulation, Electrospray, Microarray, Anisotropy**

I. Background

TRANSCRIPTION factors (TFs) are proteins which regulate the expression of genes in all living organisms. Unfortunately, the functions of all but a handful of transcription factors are known. All transcription factors possess a DNA binding domain which interacts with specific DNA sequences. The DNA binding event may either promote or inhibit transcription by RNA polymerase. Many TFs utilize regulatory mechanisms involving small molecule effectors which initiate a conformational change in the protein in order to either promote or inhibit binding to DNA, or to promote or block RNA polymerase. In-depth knowledge of metabolic regulation by transcription factors and their effectors will be extremely valuable for optimizing biofuel production, synthetic biology, bioremediation, carbon sequestration and control of biothreats and disease. *Burkholderia xenovorans* LB400 is ideal for such investigations because it has an extremely large and functionally diverse genome, is already being used for degradation of halogenated aromatic compounds and is related to several potentially dangerous pathogens (*1*).

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[1]Bioscience Division B8: Bioenergy and Environmental Science, Los Alamos National Laboratory, Los Alamos, NM.
E-mail: rshall@lanl.gov

II. Method

A systematic approach for the identification of metabolic regulatory networks was performed by utilizing FAC-MS with a Thermo Exactive electrospray LC-MS and thermal shift assays with a BioRad MyIQ real-time PCR for the identification of metabolites which may control the TF function and specificity. A consensus DNA binding sequence was determined using a protein binding microarray from Agilent, and possible operator sequences were identified in the genome of *B. xenovorans,* using the Regulatory Sequence Analysis Tools website (http://rsat.ulb.ac.be/rsat/). Finally, fluorescence anisotropy was employed for the quantification of individual DNA sequence binding affinities and for the characterization of small molecule perturbations on TF function and specificity.

III. Conclusion

Our predictions were successful in enabling the selection of a small library of metabolic compounds for screening as transcription factor effectors. FAC-MS was successful in identifying L-kynurenine, L-Phenylalanine and L-Tryptophan as possible effectors of Bxe_A0736. Thermal shift assays were successful in identifying ATP and L-kynurenine as strong binders of Bxe_A0736. The protein binding microarray has identified a degenerate consensus sequence (SVDWHHWT) which is present 4 times with regular 25-26 bp spacers within the inter-genomic sequence upstream of Bxe_A0736 and the genes which appear to be under its regulation. Additionally, this motif is present in the intergenomic DNA upstream of 31 other genes in this organism. Preliminary fluorescence anisotropy experiments suggest that L-kynurenine promotes DNA binding, while ATP inhibits DNA binding.

References

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